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Product no AS16 3939

Anti-GPA1 | Guanine nucleotide-binding protein subunit alpha 1

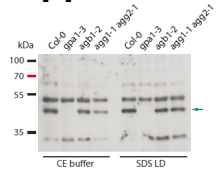
Product information

Immunogen	KLH-conjugated synthetic peptide derived from *Arabidopsis thaliana* GPA1, UniProt: P18064, TAIR: AT2G26300
Host	Rabbit
Clonality	Polyclonal
Purity	Serum
Format	Lyophilized
Quantity	50 µl
Reconstitution	For reconstitution add 50 µl of sterile water
Storage	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

Application information

Recommended dilution	1: 2000 (WB)
Expected | apparent MW	49.3 kDa
Confirmed reactivity	*Arabidopsis thaliana*
Predicted reactivity	*Amborella trichopoda, Ananas comosus, Arachis sp., Beta vulgaris, Brassica sp., Cajanus cajan, Camelina sativa, Capsella rubella, Capsicum annuum, Cicer arietinum, Coffea canephora, Cucumis melo, Daucus carota, Elaeis guineensis, Eucalyptus grandis, Eutrema sp., Fragaria vesca, Genlisea aurea, Glycine max, Glycine soja, Jatropha curcas, Malus domestica, Manihot esculenta, Medicago truncatula, Morus notabilis, Musa acuminata, Nelumbo nucifera, Nicotiana sp., Phoenix dactylifera, Phaseolus vulgaris, Populus sp., Prunus mume, Pyrus sp., Ricinus communis, Sesamum indicum, Solanum sp., Spinacia oleracea, Tarenaya hassleriana, Theobroma cacao, Vigna angularis, Vitis vinifera, Ziziphus jujuba* Species of your interest not listed? Contact us
Not reactive in	No confirmed exceptions from predicted reactivity are currently known

Application example



The samples extracted either directly with SDS loading buffer (50mM Tris-HCl pH6.8, 100mM DTT, 2%SDS, 10% glycerol, 0.025% bromophenol blue) and then denatured at 95°C for 5 min, or first extracted with CE buffer (250mM sucrose, 100mM HEPES-KOH pH 7.5, 5% glycerol, 1mM Na_2MoO_4 x $2H_2O$, 25mM NaF, 10mM EDTA, 1mM DTT, 0.5%Triton X-100, protease inhibitor cocktail) and then denatured with SDS loading buffer at 70°C for 5 min. 10 µl of proteins were loaded into each well and separated on 10% SDS-PAGE and blotted 1h to PVDF using tank transfer. Blots were blocked with Tris-buffered saline containing 0.05% Tween-20 (TBS-T) and 5% skimmed milk powder for 1h at room temperature (RT) with agitation. The blot was incubated in the primary antibodies indicated at a dilution of 1: 5 000 overnight at 4°C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed 5 times for 15 min in TBS-T with milk powder at RT with agitation. Blot was incubated in secondary antibody (Goat-Anti-Rabbit AP conjugate, Sigma) diluted 1:5000 for 2h at RT with agitation. The blot was washed as above with TBS-T without milk powder, equilibrated in AP buffer (100mM TRIS pH=9.5, 100mM NaCl, 50mM $MgCl_2$) and then developed with chromogenic detection system, before exposure to a CEA RP NEW film for 20 s.

Courtesy of Dr. Elena Petusching, Georg-August-University Goettingen, Germany

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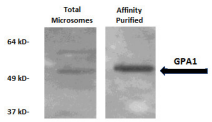

Immunodetection of GPA1 (alpha subunit of heterotrimeric G protein). Microsomes were affinity purified to isolate palmitoylated proteins using the acyl biotin exchange method1. Proteins were separated by SDS-PAGE and immunodetected with anti-GPA1. GPA1 is expected to be modified by S-acylation and was enriched in the affinity-purified fraction.

1. Wan, J., Roth, A. F., Bailey, A. O. & Davis, N. G. Palmitoylated proteins: purification and identification. Nat. Protoc. (2007). doi:10.1038/nprot.2007.225

Palmitoylated (S-acylated) proteins were affinity purified from Arabidopsis thaliana using the acyl biotin exchange method1 and separated using SDS-PAGE. Proteins were electro-transferred to PVDF membrane (Millipore, Cat. No. IVPH00010). The membrane was blocked with 5% non-fat dry milk (NFDM) in Tris-buffered saline (TBS) for two hours at room temperature. All incubations were performed with gentle agitation. The membrane was treated with primary antibody (anti-GPA1 [Agrisera, Cat. No. AS12 2370]; 1:7500 in TBS containing 5% NFDM and 0.05% Tween-20) for 2 hours. Blots were quickly rinsed twice with TBS containing 0.05% Tween (TBS-T), then washed five times for 15 minutes each with TBS-T on a shaker. The membrane was treated for 45 minutes with HRP-tagged secondary antibody (Agrisera, Cat. No. AS09 602) diluted 1:10 000 in TBS-T containing 5% non-fat dried milk. Blots were washed 2-3 times for 15 minutes with TBS-T, followed by two 15-minute washes with TBS. Immunolabeled proteins were detected with chemiluminescent detection reagent and imaged using X-ray film.

Courtesy of John McLarney under the guidance of Dr. Estelle Hrabak, University of New Hampshire, USA